CHANGES IN THE HEMOCYTE PICTURE OF GALLERIA MELLONELLA (LINNAEUS) [1]

JACK COLVARD JONES [2]

Department of Entomology, University of Maryland, College Park, Maryland 20742

In this paper differential and total hemocyte counts were obtained and combined with hemolymph volume determinations in order to estimate the changes which occur in the hemocyte picture of the wax moth *Galleria mellonella* (Linnaeus) from the eleventh through the twenty-first days of larval life, during which period the larvae pass through successive phases of feeding, crawling, spinning a cocoon, and preparing to pupate.

When reared by the method of Beck (1960), the larvae reach a fairly large size within about 10 to 11 days. During the next 10 days or so they are particularly suitable for hematological studies. Hemolymph for differential counts was collected from manually immobilized, unanesthetized, 10- to 12-day-old larvae by piercing an intersegmental membrane with a sharp needle. Hemolymph for differentials from larger larvae was conveniently obtained either by cutting a proleg or one of the protuberances on the last abdominal segment. The fresh, unfixed, and undiluted hemolymph was collected directly on a slide and a coverslip added. The cells were examined with a phase contrast microscope at × 970 and were classified using the nomenclature of Jones (1962). From 200 to 1000 cells were identified per preparation. All studies were made on larvae freshly taken from an incubator held at 34° to 35° C.

Total hemocyte counts (cells per microliter) were generally made on the first drop of hemolymph emerging from a cut proleg of both unfixed (= untreated) and heat-fixed larvae.[3] Heat-fixation consisted of immersing larvae in a water bath at 55° C. for one minute. Hemolymph was quickly drawn to the 0.25 mark of a Thoma WBC diluting pipette and then rapidly diluted with 2% acetic acid to the 11 mark. After shaking vigorously and discarding the first three drops from the pipette, a double-lined hemocytometer was filled and the cells within 5 of the one-millimeter ruled squares were counted.

Hemolymph volumes were determined using the method of Yeager and Munson (1950), that is, by injecting the larvae with 10 microliters of 1% amaranth red in saline per gram body weight. Five larvae were used for each day of study. The dye was allowed to circulate for 3 to 5 minutes and a proleg severed and the

[3] Differential and total counts were made on separately reared batches of larvae.

hemolymph collected in a capillary tube. The intensity of the color was compared to a series of known dilutions of the dye. The hemolymph volume percentages were converted into microliters.

Results

1. Differential hemocyte counts

During the last 10 to 12 days of larval life the following types of hemocytes could be easily recognized in unfixed hemolymph examined with phase microscopy: (1) prohemocytes, (2) plasmatocytes, (3) spherule cells, (4) adipohemocytes, and (5) oenocytoids, as Ashhurst and Richards (1964) have previously noted. Because so many transitional forms were seen between prohemocytes and plasmatocytes, it was very difficult or impossible to separate them accurately for quantitative work and these two types were combined into a common category which will, for convenience, be termed "plasmatocytoids." Cells seemingly transitional between plasmatocytoids and mature adipohemocytes were encountered during a definite period of larval life, and a series of counts were made in which this apparently intermediate category of cells was enumerated in addition to the other categories. These intermediate cells are termed *immature adipohemocytes.* Mitotically dividing hemocytes (probably prohemocytes) were counted and treated separately from the other categories. Adipohemocytes and spherule cells were never seen in division. The following types of hemocytes were not seen in *Galleria mellonella* larvae: granular hemocytes, cystocytes, podocytes, and vermiform cells. A few degenerating and unidentifiable hemocytes were encountered and they were so categorized in many differential counts.

Differential counts are given in Table I. During the actively feeding period (that is, from the eleventh through the fifteenth days of larval life), it is evident that (1) the plasmatocytoids ranged from 90% to 100% (with an overall mean of 96.7%), (2) adipohemocytes were consistently absent from the circulating hemolymph, (3) spherule cells varied from none to 7% and averaged 1.4%, (4) oenocytoids ranged from none to 8% and averaged 1.7%, (5) degenerating and unidentifiable hemocytes varied from none to 3%, and (6) mitotically dividing hemocytes (prohemocytes?) averaged 0.65%.

During the crawling, non-feeding, pre-cocoon-spinning period (approximately between the sixteenth and seventeenth days), a few adipohemocytes were noted in differential counts, and the number of dividing cells in such counts was reduced to about one-half that of the actively feeding period.

As soon as the larvae start to spin their cocoons, however, immature adipohemocytes suddenly increased to 12.6% and reached a maximum of 15.6% in lightly cocooned larvae and thereafter declined. Mature adipohemocytes steadily increased from 16% in the spinning period to a maximum of 57.1% in the newly formed pupae. Spherule cells rapidly declined, following the lightly cocooned period and were not observed in the pupae examined. Very few oenocytoids were seen in young pupae.

According to the differential counts, as the larvae transform into pupae, (1) plasmatocytoids decrease from about 96% to 41%, (2) immature adipohemocytes suddenly appear, (3) mature adipohemocytes steadily increase, (4) spherule cells

TABLE I

Differential hemocyte counts from unfixed last stage Galleria mellonella larvae and newly formed pupae. P = prohemocytes plus plasmatocytes (=plasmatocytoids); I = immature adipohemocytes; A = mature adipohemocytes; S = spherule cells; O = oenocytoids; D = degenerating hemocytes; U = unidentifiable hemocytes; M = mitotically dividing cells

Status	Number used	Days old	Number cells counted	Differential counts with ranges and means in parentheses (%)							
				P	I	A	S	O	D	U	M
Feeding in the medium	4	11	800	91–98 (95)	0	0	1–2 (1.4)	0–7 (2.5)	0–3	0–1	0–9 (2.5)
	4	12	1900	90–99 (96.8)	0	0	0–3 (1.4)	0–8 (1.5)	0–1	0–2	0–1 (0.2)
	5	13	2500	91–99 (95.4)	0	0	0–4 (1.4)	0–8 (2.9)	0–1	—	0–2 (0.5)
	10	13	2000	92–100 (96.5)	0	0	0–7 (2.4)	0–5 (1.1)	—	0–1	0–1 (0.25)
	5	14	1100	95–99 (97.2)	0	0	0–3 (1.3)	0–5 (1.2)	0–1	—	0–2 (0.7)
	10	14	2000	93–100 (98.1)	0	0	0–3 (0.6)	0–4 (1.3)	—	0–1	0–2 (0.3)
	5	15	2400	91–99 (96.5)	0	0	0–6 (1.5)	0–5 (1.8)	0–2	0	0–2 (0.3)
	10	15	2000	96–100 (98.0)	0	0	0–3 (1.0)	0–3 (1.0)	—	—	0–2 (0.45)
	5	16	1000	87–99 (95.5)	0	present	0–8 (2.7)	0–5 (1.7)	0–1	0–1	0–1 (0.2)
	10	16	2000	97–100 (98.4)	0	0	0–3 (1.0)	0–1 (0.6)	—	—	0–1 (0.25)
	5	17	1000	67–100 (92.7)	—	0–30 (4.8)	0–2 (0.5)	0–4 (1.9)	0	0–1	0–2 (0.6)
Spinning	10	17	2000	44–75 (59.0)	25–47 (37.9)	0–7 (0.9)	0–3 (0.9)	0–3 (1.3)	—	—	0–2 (0.55)
Early spinners	5	18	1000	45–80 (64.9)	—	15–47 (32.0)	0–6 (1.4)	0–3 (1.6)	0–1	0	0
Older spinners	5	19	1000	41–100 (80.0)	—	0–47 (15.1)	0–5 (2.2)	0–7 (2.7)	—	—	0–1 (0.3)
Lightly cocooned	3	20	600	31–95 (76.5)	—	0–68 (19.5)	0–2 (1.7)	0–4 (2.3)	0	0	0–1 (0.3)
	10	18	2000	20–52 (38.3)	35–57 (46.7)	0–42 (13.1)	0–3 (0.6)	0–3 (1.3)	—	—	0–1 (0.3)
	4	21	800	27–62 (41.0)	—	32–71 (54.9)	1–5 (2.3)	0–6 (1.7)	0–1	0	0
Densely cocooned	10	19	2000	29–61 (44.3)	2–57 (25.1)	1–56 (28.4)	0–4 (0.8)	0–6 (1.4)	—	—	0–1 (0.1)
	10	20	2000	31–63 (43.9)	0–19 (7.2)	29–62 (46.6)	0–3 (1.0)	0–3 (1.3)	—	0	0–1 (0.05)
	6	19–21	1200	18–47 (34.7)	—	51–79 (63.0)	0–2 (0.6)	0–5 (1.7)	0–1	0	0–1 (0.08)
Newly formed pupae	10	21	2000	21–66 (42.4)	0–3 (0.4)	34–79 (57.1)	0	0–1 (0.1)	—	—	0

decrease after larvae are lightly cocooned, (5) oenocytoids decrease, and (6) dividing cells steadily decline.

Out of 56 cases where records were kept, 75% of the dividing hemocytes were in metaphase, 23.2% in telophase, and 1.8% were in anaphase. Prophases could not be recognized with the methods used.

2. Total hemocyte counts

Total hemocyte counts (THC) were made daily from the thirteenth through the twenty-second days of life, from 150 unfixed and from 139 heat-fixed larvae. As reported in Table II, unfixed THC are more variable (4.9- *versus* 3.2-fold mean variation), and were consistently and significantly lower than heat-fixed counts at greater than the 95% level (that is, twice the standard errors of daily unfixed and

TABLE II

Daily total hemocyte counts with standard errors from unfixed and heat-fixed
Galleria mellonella larvae

Days old	No. used	Cells per microliter			
		Unfixed		No. used	Heat-fixed
13	5	20,336	± 3360	5	50,448 ± 4640
14	11	21,933	± 1804	10	49,686 ± 3200
15	16	23,704	± 2210	10	42,992 ± 3480
16	16	31,045	± 2635	15	53,504 ± 1661
17	22	25,252	± 1531	22	43,316 ± 2869
18	16	27,609	± 1880	16	54,042 ± 4425
19	17	27,053	± 2334	16	56,432 ± 5460
20	15	31,925	± 2779	15	48,724 ± 3739
21	16	37,744	± 2320	15	61,752 ± 3515
22	16	35,613	± 3545	15	54,672 ± 3488
	Mean	28,221.4 ± 2440		Mean	51,556.8 ± 3648

heat-fixed counts did not approach an overlap on any of the days studied). In both types of counts the numbers of cells per microliter of hemolymph increase as larvae proceed toward the pupal stadium, and in both the increase is about the same (*i.e.,* from 17,000 to 18,000 hemocytes/microliter). In both, the most variable counts were obtained on the seventeenth day. The unfixed counts from 20- to 22-day-old larvae are comparable to the value of 33,200 hemocytes per microliter reported by Stephens (1963) for larvae which were reared by a different method and developed much more slowly. However, the consistently and significantly higher counts from heat-fixed *Galleria* are definitely not in agreement with Stephens' statement that heat fixation does not alter the counts.

Unfortunately only a few records were made on the status of the various larvae during the above period. The records which were obtained, however, were as follows. In 6 newly emerged larvae the unfixed THC averaged 22,666. During the spinning of the cocoon, 32,584 cells were found per microliter from 10 unfixed larvae. Three larvae from light cocoons had 33,067 cells per microliter. Unfixed

THC from four larvae taken from dense cocoons amounted to 44,620 cells, and counts from three prepupae came to 17,867 hemocytes. These few data suggest that the THC increases as larvae spin their cocoons, that there is a further and greater increase after they complete the cocoon-spinning process, and that the counts begin to decrease in the prepupae. The counts from densely cocooned larvae and pharate pupae are strikingly higher than those found by Shrivastava and Richards (1965), possibly because they used chilled material and excluded the first two drops of hemolymph.

3. Hemolymph volumes

Hemolymph volume determinations were made from 15- to 22-day-old unfixed *Galleria,* all from a single batch of individuals. The larvae were all still feeding in the medium on the seventeenth day. On the eighteenth day, four out of 5 larvae had already spun a light cocoon. By the twenty-second day, they had all pupated. As presented in Table III, the hemolymph volumes when viewed as percentages

TABLE III

Hemolymph volumes of 15- to 22-day-old Galleria mellonella (unfixed)

Age in days	Status	% Body weight		Calculated microliters	
		Range	Mean	Range	Mean
15	Feeding	33–36	34.2	36.5–54.0	43.1
16	Feeding	33–36	33.6	63.2–73.6	67.7
17	Feeding	33–36	34.2	48.1–72.2	59.4
18	Lightly cocooned*	28–35	31.6	40.1–53.7	46.7
19	Medium cocoon	29–33	31.4	41.5–7.16	58.1
20	Dense cocoon	19–31	26.0	29.9–57.7	42.4
21	Dense cocoon	16–32	24.8	27.8–46.3	35.8
22	Pupae	16–18	16.4	17.0–24.5	18.8

* Only one of the larvae was beginning to spin a cocoon.

of the body weight remain level at about 34% during the feeding period and then gradually decrease to less than 16.4% in newly formed pupae. Considered as microliters, hemolymph volumes of feeding larvae averaged 56.7 and tended to decline thereafter. Hemolymph volumes of cocooning or cocooned larvae averaged 45.7 microliters. With pupation, the volumes obtained were from less than 17 to 24.5, with a mean of about 19 microliters.

Hemolymph volumes were also made from a subsequent batch of 10 unfixed and 10 heat-fixed larvae of the same age. The hemolymph volumes were identical (45.5 microliters).

4. Calculated hemocyte populations

The preceding information can be combined to indicate changes in the hemocyte population within the entire insect. Thus, when THC values are multiplied by the hemolymph volumes, it can be calculated that, at the 95% level, there are from 831,140 to 2,458,525 hemocytes available in the circulating hemolymph of unfixed

larvae from the fifteenth through the twenty-first days, with means fluctuating around 1,456,000 (Table IV). The daily mean hemocyte population from the fifteenth through the twenty-first days varied by a factor of only 1.2-fold. The data suggest that the circulating hemocyte population in unfixed larvae remains about the same up to pupation itself, at which time there is a very striking and significant decrease (at the 95% level) so that more than one-half of the hemocyte population is no longer circulating in unfixed newly formed pupae.

5. Calculated changes in the components of the hemocyte population

Calculations on the components of the hemocyte population from the fifteenth through the twenty-second days are presented in Tables V and VI. Assuming that these data give a reasonable approximation towards the real situation, the following estimations can be made. (1) During the feeding period, plasmatocytoids averaged 1,490,569 (95% range = 802,050–2,419,189) and during cocooning they aver-

TABLE IV

Calculated circulating hemocyte populations in unfixed 15- to 22-day-old
Galleria mellonella

Age in days	Status	Range at 95% level	Mean
15	Feeding	831,140–1,212,144	1,021,642
16	Feeding	1,744,967–2,458,525	2,101,746
17	Feeding	1,318,086–1,681,852	1,499,969
18	Light cocoon	1,113,748–1,464,932	1,289,340
19	Medium cocoon	1,300,568–1,842,990	1,571,779
20	Dense cocoon	1,117,961–1,589,279	1,353,620
21	Dense cocoon	1,185,123–1,517,347	1,351,235
22	Pupae	536,232– 802,816	669,524

aged 563,308 (95% range = 411,238–816,445), amounting to an average decrease of 927,261 cells (95% range = 390,812–1,602,744). With pupation, there was a further average loss of 185,000 cells (95% range = 183,876–186,125), amounting to an average total loss of 1,112,261 plasmatocytoids (95% range = 574,688–1,788,869) as *Galleria* larvae transform into pupae. (2) From 520,000 to 684,123 immature and 145,900 to 192,000 matured adipohemocytes appeared in the hemolymph of lightly cocooned larvae, a range of 666,000 to 876,000 (average 771,025) cells containing lipid inclusions. Immature adipohemocytes decreased by 439,627–569,695 (average 504,661) cells within the densely cocooned larval insect. With pupation, there was a further decrease of 78,348–111,217 cells (average 184,996), thus amounting to 517,975–680,912 immature adipohemocytes deleted during the larval-pupal molt and ecdysis. (3) After mature adipohemocytes first appeared in the hemolymph, they increase to a maximum of 955,929. Between the eighteenth and twenty-first days, 600,726–764,023 (average 682,375) mature adipohemocytes were formed, on each of which days they increased by 151,000–332,000. After pupation, 440,000–498,000 adipohemocytes were no longer circulating. (4) During larval life, spherule cells averaged 16,950 and they were not observed in the pupae examined. They reached a maximum on the sixteenth day, that is, before the

TABLE V

*Calculated mean changes in the components of the hemocyte population of unfixed
15- through 22-day-old Galleria mellonella*

Age in days and status	Numbers of circulating hemocytes					
	Plasmatocytoids	Adipohemocytes		Spherule cells	Oenocytoids	Dividing hemocytes
		Immature	Mature			
15 Feeding	985,884	0	0	15,325	18,389	3,065
15 Feeding	1,001,209	0	0	10,012	10,012	4,597
16 Feeding	2,007,167	0	0	56,747	35,730	4,203
16 Feeding	2,068,118	0	0	21,017	12,610	5,254
17 Feeding	1,390,471	0	71,998	7,499	28,499	9,000
18 Light cocoon	493,817	602,122	168,903	7,736	16,761	3,868
19 Dense cocoon	696,298	394,516	446,385	12,574	22,005	1,572
20 Dense cocoon	594,239	97,461	630,787	13,536	17,597	667
21 Dense cocoon	468,878	—	851,278	8,107	22,971	1,081
22 Pupae	283,878	2,678	382,298	0	669	0

larvae began to spin a cocoon. (5) Oenocytoids fluctuated from 10,012 to 35,730 during larval life, with an overall mean of 20,508 and, like the spherule cells, attained a maximum on the sixteenth day. (6) From 559 to 10,091 hemocytes apparently divide in the hemolymph from the fifteenth through the twenty-first days of life, the greatest number being present on the seventeenth day. Since it is not known whether mitotic divisions occur throughout the day and since the duration of the mitotic cycle is unknown, it is not possible to make correlations between mitoses and changes in the hemocyte population.

In their radioautographic study, Shrivastava and Richards (1965) showed that plasmatocytes of *Galleria* transform into adipohemocytes within 24 hours, and the present hemocyte population calculations were examined to see if the changes in the population of plasmatocytoids could be correlated with adipohemocyte popula-

TABLE VI

Calculated ranges at 95% level of plasmatocytoids and adipohemocytes

Age in days and status	Plasmatocytoids	Adipohemocytes	
		Immature	Mature
15 Feeding	802,050–1,169,719	0	0
15 Feeding	814,517–1,187,901	0	0
16 Feeding	1,666,443–2,347,891	0	0
16 Feeding	1,717,047–2,419,189	0	0
17 Feeding	1,221,866–1,559,077	0	58,170– 74,836
18 Light cocoon	426,565– 561,069	520,120–684,123	145,901–191,906
19 Dense cocoon	576,152– 816,445	326,443–462,590	369,361–523,409
20 Dense cocoon	490,785– 697,693	80,493–114,428	520,970–740,604
21 Dense cocoon	411,238– 526,519		746,627–955,929
22 Pupae	227,362– 340,394	2,145– 3,211	306,188–458,408

tion changes. The data in Table VII show the changes in the populations of plasmatocytoids and of immature and mature adipohemocytes in terms of the 95% ranges with the means in parentheses. (1) There is no correlation of changes in the two populations between the sixteenth and seventeenth days (that is, 7.5 to 10.5 times more plasmatocytoids disappear than adipohemocytes appear). (2) If it is assumed that all of the mature adipohemocytes already in circulation on the seventeenth day (71,998 cells) remain in circulation on the eighteenth day, then 96,905 new adipohemocytes would need to be formed from plasmatocytoids. Between the seventeenth and eighteenth days, 896,654 plasmatocytoids disappeared and 771,025 adipohemocytes appeared which leaves a deficit of 125,629 plasmatocytoids unaccounted for in a population of 1,264,842 (an error of 10%). This is interpreted to mean that many (about 64%) plasmatocytoids transform into immature and mature adipohemocytes between the seventeenth and eighteenth days. (3) Between the eighteenth and nineteenth days the plasmatocytoids increased by 202,481 cells, the immature adipohemocytes decreased by 207,606 cells, while mature adipohemocytes increased by 277,482 cells. If all of the mature adipohemocytes of the eight-

TABLE VII

Estimated increases and/or decreases in populations of plasmatocytoids, immature and mature adipohemocytes in unfixed Galleria mellonella. Ranges at 95% level; means in parentheses

Between days	Plasmatocytoids	Adipohemocytes	
		Immature	Mature
16–17	−495,181 to 860,112 (−677,647)	—	+ 58,170 to 74,836 (+ 71,998)
17–18	−795,301 to 998,008 (−896,654)	+520,120 to 684,123 (+602,122)	+ 87,731 to 117,070 (+ 96,905)
18–19	+149,587 to 255,376 (+202,481)	−193,677 to 221,533 (−207,606)	+223,460 to 331,503 (+277,482)
19–20	− 85,367 to 118,752 (−102,059)	−245,950 to 348,162 (−297,055)	+151,609 to 217,195 (+184,402)
20–21	− 79,547 to 171,174 (−125,361)	—	+215,325 to 225,657 (+220,491)

eenth day remained in circulation on the nineteenth day, then 277,482 new mature adipohemocytes would need to be formed. If all 207,606 immature adipohemocytes which disappeared from the circulation between the eighteenth and nineteenth days were transformed into mature adipohemocytes, this would leave only 69,876 mature adipohemocytes unaccounted for on the nineteenth day. This appears to be an excellent correlation and implies that about 34% of the 168,903 immature adipohemocytes in circulation on the eighteenth day transform into mature cells by the nineteenth day. (4) Between the nineteenth and twentieth days the populations of both plasmatocytoids and immature adipohemocytes appear to decrease simultaneously and far more immature adipohemocytes disappear than new ones form. No correlations could be detected then between the various hemocytes between the nineteenth and twentieth days. (5) Between the twentieth and twenty-first days the plasmatocytoids decreased by 125,361 cells and mature adipohemocytes increased by 220,491 cells. If all 530,787 mature adipohemocytes of the twentieth day remained in circulation and all of the circulating immature adipohemocytes transformed into mature cells by the twenty-first day, this would still leave 123,030 mature adipohemocytes unaccounted for. If most of the 125,361 plasmatocytoids which disappeared between the twentieth and twenty-first days transformed into

mature adipohemocytes, this would account for the deficit and make an almost perfect correlation.

Considering the many sources of error in calculations such as these, it is remarkable that it was possible to make any correlations, and impressive that three out of five of them appear so close. These correlations suggest (1) that many plasmatocytes transform into both immature and mature adipohemocytes between the seventeenth and eighteenth days when larvae are spinning a cocoon, (2) that between the eighteenth and nineteenth days, when the larvae are cocooned, mature adipohemocytes are largely being formed by maturation of immature adipohemocytes, and (3) that in pharate pupae mature new adipohemocytes are being formed from both immature adipohemocytes and from plasmatocytoids between the twentieth and twenty-first days.

DISCUSSION

In *Prodenia* larvae Yeager (1945) recognized and counted separately adipohemocytes (his "spheroidocytes") and granular hemocytes (his "cystocytes"). Jones (1959) pointed out that when the adipohemocytes of *Prodenia* matured they closely resemble the granular hemocytes. Yeager (1945) suggested that the adipohemocytes were derived from prohemocytes and, since he observed mitoses among adipohemocytes, they might also be considered as a self-perpetuating line of cells in this insect. He suggested that the granular hemocytes of *Prodenia* were derived from plasmatocytes. He observed mitoses among granular hemocytes, though less commonly than in the plasmatocytes and adipohemocytes. In *Bombyx,* Nittono (1960) apparently combined Yeager's cystocytes and spheroidocytes into a common category which he designated granular hemocytes. Earlier, Jones (1959) had suggested that Yeager's "cystocytes" were comparable to the granular hemocytes of other insects. In some insects, cells termed granular hemocytes are quite distinct from both plasmatocytes and adipohemocytes: for example, in the blood-sucking bug, *Rhodnius prolixus* (Jones, 1965), the granular hemocytes possess many uniform discrete inclusions and are not derived from plasmatocytes and are not related to them. In *Sarcophaga,* changes in the population of cells termed granular hemocytes (Jones, 1956) cannot be correlated with changes in the population of plasmatocytes (Jones, unpublished data). The "granular hemocytes" of *Bombyx* are present in large numbers in one- to three-day-old larvae of the fifth stage and they were frequently observed in mitotic division (p. 262) by Nittono. The data in Nittono's Tables 4 and 6 were combined so that estimates could be made of the components of the hemocyte population with time in both males and females of the fifth stage. No correlations at all could be found between the populations of plasmatocytoids and adipohemocytes at any time. Dr. Nittono (personal communication) has confirmed this. Can the granular hemocytes of *Bombyx* and *Sarcophaga* which are present throughout larval life and which do not appear to be derived from plasmatocytes be compared with the adipohemocytes of *Galleria* when the latter appear *only* near the end of larval life, do *not* divide, and *are* derived from plasmatocytes? Granular hemocytes and adipohemocytes may both be phagocytic and yet very different in their origins. There is no doubt that the granular hemocytes in *Rhodnius* are *not* comparable morphologically or physiologically to the adipohemocytes of *Galleria.* Until considerably more information is available

concerning the granular hemocytes and adipohemocytes, separate terms should be retained. From the evidence now available it would seem that granular hemocytes of some insects are *not* derived from plasmatocytes whereas adipohemocytes of a number of the Lepidoptera arise by direct transformation of circulating plasmatocytes.

In *Prodenia,* plasmatocytoids decline from 86.3% in the first instar larvae to 34.2% in prepupae (calculations from Yeager's data, 1945). Granular hemocytes appeared first in fourth-stage larvae and increased to 28% just before pupation (Yeager, 1945). Adipohemocytes increased from 2.5% in first stage larvae to 38.6% in prepupae. Spherule cells reached their maximum (43.4%) in third-stage larvae and declined to 6% the day before pupation (Yeager, 1945). During the last three days before pupation of *Prodenia,* plasmatocytoids decreased from 41.7% to 17.8%, adipohemocytes increased from 33.9% to 43.9%, granular hemocytes increased from 5.2% to 28%, and oenocytoids decreased from 5.1% to 1.8% (Yeager, 1945).

In last-stage *Bombyx* larvae, plasmatocytoids reached a peak of 67.3% on the seventh day and declined to 27.3% just before pupation. In the J 122 X C strain, the granular hemocytes averaged 53.4% from the third through the fifth larval stages and definitely increased near the end of each stadium. During the first eight days of the last larval stage they averaged 45.7%, and during the last four days they averaged 64.1% (Nittono, 1960).

Galleria resembles *Prodenia* and *Bombyx* in that plasmatocytoids decrease and that hemocytes with many polysaccharide and/or lipid or other types of inclusions increase prior to pupation. *Galleria* differs significantly from *Prodenia* and *Bombyx* in that their hemocytes with many polysaccharide and/or lipid or other types of inclusions do not appear in the hemolymph for the first five days of the last larval stadium. The hemocytes with lipid inclusions in *Bombyx* apparently are not derived from plasmatocytes (at least no correlations between changes in these two components of the population were detectable), whereas in *Galleria* there is radioautographic evidence that hemocytes with lipid inclusions are derived from plasmatocytes, and in three out of five cases it was possible to detect reasonably close reciprocal correlations between the changes in these two cell types.

Summary

1. The hemocytes of *Galleria mellonella* (Linnaeus) larvae were identified and differentially counted in unfixed hemolymph with phase microscopy. The numbers of hemocytes per microliter of hemolymph were obtained from both unfixed and heat-fixed larvae. Hemolymph volumes were determined by the amaranth red method. These studies were made to determine what changes in the hematology occur as the last stage larvae pass through distinctive phases in transforming into pupae.

2. In differential counts, plasmatocytoids decrease, immature adipohemocytes suddenly appear, and mature adipohemocytes steadily increase. Spherule cells, oenocytoids and dividing hemocytes decrease as *Galleria* larvae develop into pupae.

3. The numbers of hemocytes per microliter of hemolymph increase as *Galleria* larvae proceed towards the pupal stage in both unfixed and heat-fixed animals. Counts were always significantly higher in heat-fixed than in unfixed larvae.

4. The hemolymph volume is the same in both unfixed and heat-fixed larvae. The hemolymph volume declines from about 34% (56.7 microliters) in precocoon-spinning larvae to less than 16.4% (19 microliters) in newly formed pupae.

5. It is estimated from the various data presented that an average of 1,456,000 hemocytes remain in circulation within the hemocoele of unfixed larvae from the fifteenth through the twentieth days of life, and that with pupation more than one-half of these cells fall out of circulation.

6. In three out of 5 cases it was possible to correlate decreases in the plasmatocytoid population with increases in adipohemocytes. It is suggested that during the spinning of a cocoon plasmatocytoids transform into both immature and mature adipohemocytes, that when the larvae are densely cocooned mature adipohemocytes are largely formed by the maturation of immature adipohemocytes, and that in pharate pupae new mature adipohemocytes are derived from both immature adipohemocytes and plasmatocytoids.

7. The hemocyte picture of *Galleria* is compared to that of *Prodenia* and *Bombyx*. In all three of these Lepidoptera the plasmatocytoids decrease and the hemocytes with many polysaccharide and/or lipid or other types of inclusions increase prior to pupation. *Galleria* differs from the other two species in that their hemocytes with lipid or other inclusions do not appear until about the sixteenth or seventeenth days of larval life, do not divide, and in many cases are derived from circulating plasmatocytes.

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